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Integrated on-chip photodetection of intracellular calcium in response to the activation of G-protein coupled receptors

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Abstract

Microfabricated amorphous silicon photodiodes have been used to monitor the increase of fluorescence in living cells following calcium release into the cytosol in response to G-protein coupled receptors (GPCR) activation. A signal acquisition system was developed, enabling real-time monitoring of the calcium fluxes by fluorescence. Calibration of the emission light versus calcium concentration demonstrates the possibility of single cell monitoring with this setup. The developed system enabled the detection of 0.5 mM agonist-induced calcium fluxes with accuracy comparable to traditional optical systems.

Keywords: GPCR; fluorescence; amorphous silicon; calcium; integrated system, PDMS

1. Introduction

G Protein Coupled Receptors (GPCRs) are one of the largest known families of cell receptors, with around 1000 GPCR-like sequences identified in the human genome. These highly specialized membrane proteins play a key role in signal transduction, converting changes in extracellular information into intracellular functions. The key role played by GPCRs in many physiological or disease-related processes

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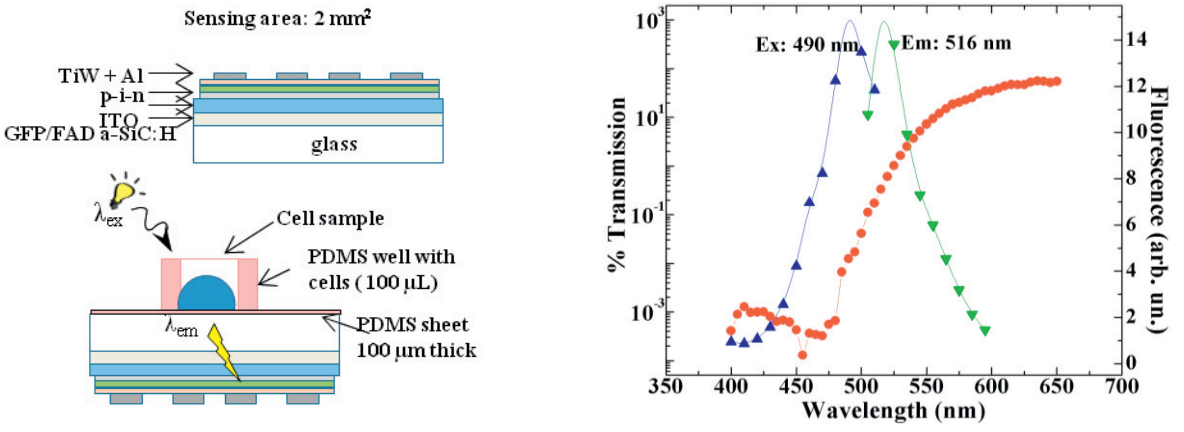
has made them one of the favorite targets of the pharmaceutical industry. Indeed, approximately 30% of the approved drugs in the market selectively target members of this family [1, 2] .

The identification of new compounds acting on GPCRs relies on the recording of the average signal from thousands of cells upon addition of a candidate drug target. Typically, changes in the intracellular levels of key elements in the signaling cascade are monitored using fluorescence read-out systems such as microscopy or CCD cameras [1]. To increase the throughput in GPCR drug discovery programs, several strategies are being pursued to miniaturize the cellular assay platforms. However, a major challenge of the miniaturization process is the scaling-down of the optical apparatus. In this work, this issue is addressed by developing cell chips based on microfluidics with integrated photodiodes for signal acquisition. These photodiodes are characterized by high photosensitivity, low dark current and high frequency response. In particular, thin film photodiodes based on hydrogenated amorphous silicon (a-Si:H) are compatible with microfabrication techniques and consequently easily integrated "on-chip" for acquisition of the optical signal [3]. For proof-of concept studies, HEK 293T cell lines endogenously expressing the Muscarinic M1 GPCR were chosen as the biological model. Activation of the M1 receptor is monitored by following the rise in intracellular calcium (iCa^{2+}) upon addition of an agonist to the cell culture.

2. Experimental Methods

The photodiodes were microfabricated on glass after deposition of a 2 µm amorphous silicon carbon-alloy (a-SiC:H) filter for excitation light rejection. The bottom contact is a 100 nm layer of indium tin oxide (ITO), a transparent conductor. The a-Si:H p-i-n photodiodes consists of 20 nm n+-a-Si:H, 500 nm intrinsic a-Si:H and 20 nm p+-a-Si:H. The top contact (TiW + Al) was defined with a physical mask with an area of 2 mm^2 which represents the actual sensing area (Figure 1 (a)).

The optical detection relies on a custom-designed transimpedance amplifier with a conversion ratio of $2x10^{10}$ V/A and a minimum sensitivity of 100 fA. The signal is sampled and averaged 600 times per second by a 10-bit precision PIC Microcontroller from Microchip Technology (model 18f4550). Serial communication between the microcontroller and the acquisition software is performed via USB.



(a) (b)

Fig. 1. (a) Schematic cross section of the photodiode (top) and the measurement setup (bottom). (b) Filter transmitance spectrum (red) overlapping the emission and the excitation spectra of the Fluo4 die. The light transmitted at 516 nm (λ_{em} Fluo4) is two orders of magnitude higher than the transmitted light at 490 nm (λ_{ex} Fluo4).

Polydimethylsiloxane (PDMS) wells were fabricated by mixing the polymer and catalyst in 10:1 ratio, poured into a Petri dish and cured for 2 hours at 70ºC. The cured PDMS was cut into 1 x 1 cm squares and punched to form 0.6 cm diameter wells. PDMS sheets of 100 µm thickness were fabricated by spin-coating the polymer on cleaned silicon wafers and cured for 2 hours at 70ºC. The sheets were used to seal the wells by performing Corona discharge ionization for 10 s. The wells were pre-coated with fibronectin (100 µg/ml in H_2O), for 1 h at 37 ºC. For the GPCR activation assays, 50 x10^3 cells HEK 293T cells in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% Pen/Strep were seeded (100 µL) on the fibronectin-coated wells and allowed to adhere for 1 h at 37ºC in an humidified 5% CO_2 atmosphere. Following adhesion, the cells were stained with the calcium sensitive fluorophore Fluo4 (5 µM in 50 µL of HEPES/HBSS buffer) for 1 h, washed with buffer and placed immediately on top of the photodiodes. Different concentrations (0, 0.5 and 1 mM in 50 µL), of the agonist carbachol were added to the system and the change in the diode photocurrent was monitored in real time.

3. Results and Discussion

Activation of GPCR type M1 receptor triggers the activation of phospholipase C (PLC) signaling pathway with the consequent release of innositol-3-phosphate (IP3) into the cytoplasm. The later in turn activates calcium sensitive channels present at the endoplasmic reticulum with the consequent release of Ca^{2+} into the cytosol [2]. The monitoring of the intracellular calcium fluxes can be accomplished by the use of fluorophores that show enhanced fluorescence upon calcium binding such as Fluo4. Optimization of incubation times assures a low compartmentalization of the fluorophore and provides a sensitive methodology to follow calcium concentration in the cytoplasm. Upon addition of an agonist, a positive signal is characterized by a steep rise in cells fluorescence, followed by a slow decay as desensitization of the GPCR occurs and calcium levels in the cytoplasm are restored to basal values (Figure 2).

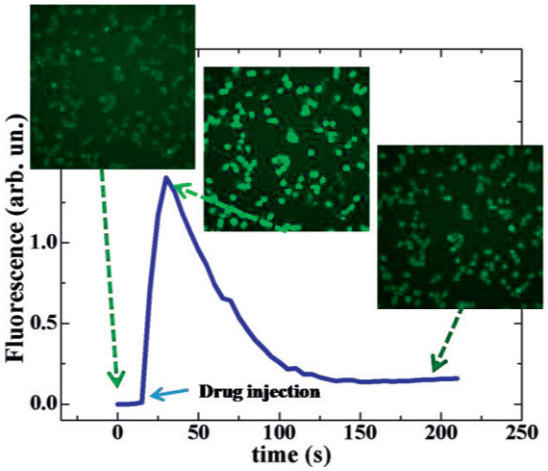
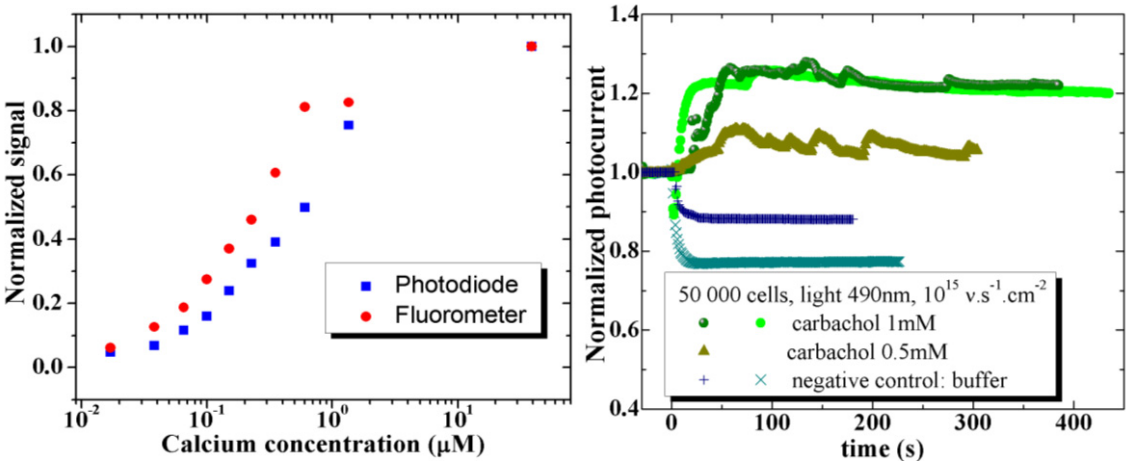


Fig. 2. Addition of 1 mM carbachol triggered the activation of the endogenous receptor M1, promoting the release of Ca^{2+} into the cytosol. Consequently, the cell fluorescence increased as detected by fluorescence microscopy. Insets show the corresponding images.

The ability to detect Ca^{2+} with the photodiode setup was assessed using calcium standard solutions (0-39 µM) incubated with Fluo4 salt. Results in figure 3 (a) demonstrate calcium detection for concentrations as low as 0.017 µM, equivalent to single cell resolution. Moreover, sensitivity and dynamic range of the photodiode setup are similar to those of conventional fluorometric assays.



(a) (b)

Fig. 3. (a) Comparison of normalized light intensity induced by different calcium concentration solutions incubated with Fluo4 with a conventional fluorometer and a microfabricated photodiode.(b) Real-time measurement of the photocurrent resulting from induced calcium fluxes for different carbachol concentrations.

For monitoring GPCR activation, the cell-containing PDMS wells were placed on top of the photodiode and stimulated with carbachol. Figure 3 (b) shows, in real time, the normalized diode photocurrent after carbachol insertion for different sets of experiments. An increase in photocurrent followed by a slow decay is observed when the agonist is added to the system. Moreover, the peak intensity is proportional to the agonist concentration. In contrast to the microscopy results (figure 1), the response signal presents a wavy behavior which may suggest the contribution to the fluorescence of independent cell clusters responding at different times.

4. Conclusions

The calcium dynamics observed upon activation of the M1 muscarinic GPCR were detected using the microfabricated a-Si:H p-i-n photodiodes. On-going work is focusing on the device miniaturization and further integration with microfluidics, which are envisaged to increase sensitivity and assay throughput.

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References

[1] Schlyer S, Horuk R. I want a new drug: G-protein-coupled receptors in drug development. *Drug Discov Today* 2006; **11**:481-493

[2] Lagerstrom, MC, Schioth HB. Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat Rev Drug Discov* 2008; **7**: 339-357

[3] Conde JP, Pimentel AC, Pereira AT, Gouvêa A, Prazeres DMF, Chu V. Detection of molecular tags with an integrated amorphous silicon photodetector for biological applications. *J Non-Cryst Solids* 2008; **354**:2594-2597